ACTIVITY OF PERA SAFE ™ AGAINST *BACILLUS ANTHRACIS* SPORES

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ABSTRACT

Fast and effective decontamination of areas contaminated with *Bacillus anthracis* spores after a successful bio-terrorist attack proves to be a challenging task. There exist a variety of disinfectants that can inactivate *Bacillus anthracis* spores; however, most of them have negative side effects, such as equipment corrosion and environmental toxicity. The investigation described here shows that Pera Safe™ has high sporocidal activity on *Bacillus anthracis* spores within 20 minutes, while being safe for use with minimal impact on the environment.

INTRODUCTION

Bacillus anthracis is one of the main organisms that can be used in biological warfare or by bio-terrorists. This pathogen, in the form of spores, is characterized by considerable resistance to external factors, as is shown by its survival in the natural environment for years. *Bacillus anthracis* spores that are used as a biological weapon will result in environmental contamination that eventually must be decontaminated. Most of the disinfectants currently used to inactivate these spores are very corrosive, thus limiting the scope of their application. The purpose of this study was to estimate the sporicidal properties of a preparation of Pera Safe™ against *Bacillus anthracis* spores. A search of the literature showed no previous work with this material.

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MATERIALS AND METHODS

For this study, a suspension of *Bacillus anthracis* spores, strain Sterne 34 F2, was used. The Pera Safe™ preparation (series 2194) was manufactured by Antec International and was supplied by Naturan LTD, Warsaw, Poland.

Spore suspension preparation: 200 ml of media, consisting of brain- heart agar with the addition of yeast extract and MnSo4 (0.1 g/l), was set in a level Roux bottle and was inoculated with 5 ml of a 24 hour broth bouillon culture of *Bacillus anthracis* (BA), strain Sterne 34 F2, and incubated for 72 hours at 37^{o} C. The culture surface was then washed with Ringer's solution (dilution 1:4), filtered through sterile gauze, and centrifuged five times at 5-7,000 revolutions per minute. After each centrifugation, the precipitate was washed with sterile PBS. The washed spores were suspended in sterile, distilled water until a titer of $1.5x10^{7}$ spores/ ml was obtained. The number of spores was determined in a Thoma chamber using phase-contrast microscopy.

Preparation of the PeraSafe™ solution. A solution of a 1.62% concentration was prepared in 500 ml flasks with sterile, distilled water at a of temperature 35^{o} C. After dissolving, the solutions were left for 1 hour at room temperature. Immediately before testing, 1% bovine albumin (Serva 11925) was added to one of the flasks. This is a standard load of organic substances used in investigations of preparations for the disinfection of instruments.

Determination of sporicidal activity. Suspensions of BA spores with concentrations between $1.5x10^{7}$ spores/ml and $1.7x10^{4}$ spores/ml were used for testing the PeraSafe™ preparation. The suspensions were centrifuged, and then 10 ml of the PeraSafe™ solution was added to each of the precipitates, respectively.

Concurrently, the same investigation was performed using the sporicidal preparation with a 1% bovine albumin supplement. The samples were mixed thoroughly and left at room temperature for 20, 40, 80, 160 and 240 minutes, respectively. After the measured time elapsed, the suspension was centrifuged, washed, and the sporicidal activity was established by incubation of 100 ul of each of the samples on blood agar.

RESULTS AND DISCUSSION

PeraSafe™ is a preparation in the form of powder containing sodium perborate, TAED, corrosion inhibitors, stabilizers, and dye. After dissolving the powder in water, supra-octane ions are liberated. This powder is used for general disinfection and shows bactericidal, fungicidal and virocidal properties.

The results are shown on Table 1. After 20 minutes, the solution of PeraSafe™ totally inactivates $5x10^{4}$ cfu/ml of *Bacillus anthracis* spores regardless of whether or not they are in the presence of bovine albumin. Higher concentrations of spores require a longer contact time, as is shown by the fact that a suspension of $1.5x10^{6}$ cfu/ml required 160 minutes for 100% inactivation. A concentration of $1.5x10^{6}$ spore/ml required at least 80 minutes of reaction time, irrespective of the presence of bovine albumin.

TABLE 1. Activity of Perasafe™ Against *Bacillus Anthracis* Spores.

PeraSafe™ contact time (min.)	**Spore Concentration (cfu/ml)**							
	1.5×10^7		**1.5×10^6**		**1.5×10^5**		**1.5×10^4**	
	Without albumin	**With albumin**	**Without albumin**	**With albumin**	**Without albumin**	**With albumin**	**Without albumin**	**With albumin**
20	$3{,}0 \times 10^2$	$5{,}4 \times 10^2$	$2{,}7 \times 10^1$	$4{,}0 \times 10^1$	0	$2{,}0 \times 10^1$	0	0
40	$1{,}1 \times 10^2$	$2{,}1 \times 10^2$	$1{,}2 \times 10^1$	$2{,}0 \times 10^1$	0	0	0	0
80	$2{,}7 \times 10^1$	$5{,}0 \times 10^1$	0	0	0	0	0	0
160	0	0	0	0	0	0	0	0
240	0	0	0	0	0	0	0	0

The results suggest that the PeraSafe™ preparation can be considered as one of the best products acting against BA spores. Currently, the following preparations are typically used for decontamination: formaldehyde, glutaraldehyde, hydrogen peroxide, peroxyacetic acid, chloramine, chlorine water(1,2,3,4,5). However, chlorine solutions corrode metals, oxidize rubber, and are rapidly neutralized by organic substances. Formaldehyde and glutaraldehyde solutions are harmful for the skin and respiratory tract. Likewise, one needs long exposure times for effective decontamination with these preparations (up to a few hours), considerably longer than PeraSafe™.

Pera Safe acts rapidly and effectively, even in cases of considerable contamination with BA spores. This preparation is not only safe to use but also is characterized by a pleasant odor.

CONCLUSIONS

PeraSafe™ inactivates *Bacillus anthracis* spores. The effectiveness of the sporicidal activity depends on the contact time and the concentration of spores. This preparation is safe for the environment.

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